CYTOLOGICAL OBSERVATIONS ON HYPOXIS:

II. POLLEN GERMINATION, POLLEN TUBE GROWTH AND HAPLOID CHROMOSOME NUMBERS IN SOME *HYPOXIS* SPECIES

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Abstract

Different methods were tried for pollen germination and metaphase was arrested in some Hypoxis species. *Hypoxis filiformis* has 7 chromosomes in the nuclei of the pollen tubes, and the same number was recorded in an unidentified species. In *H. rooperi* there is great variation in chromosome number in different pollen tubes (43 to 58) and *H. acuminata* (n = 18) shows some pollen tubes with 20 chromosomes.

Polyploidy has played an important role in the phylogeny of *Hypoxis* species and the chromosome number is often so high (over 80) that analysis of root tip preparations is almost impossible. In order to determine chromosome numbers it was decided to study the cell division of the generative nucleus in pollen tubes. For this reason pollen germination experiments were done, and it was combined with metaphase arrest to achieve greater separation of the chromosomes.

In a previous paper (Wilsenach, J. S. A. B., 33,1) abnormal meiosis was reported in the polyploid *Hypoxis* species, which led to the assumption that the pollen grains of such plants vary in chromosome number. This assumption can also be tested by studying the chromosome numbers in different pollen tubes of one plant.

Table 1

Sucrose concentration	Maximum tube length after 2 hours	Maximum tube length after 15 hours
2½%	0	—
4	0	—
5	70μ	—
8	60	—
12	110	—
15	220	240μ
20	200	—

METHODS AND RESULTS

The pollen used was that of *Hypoxis rooperi.*

A. *The pollen germination method of Necomer* (1938).

A sugar-agar medium is used, giving the following results (incubation at room temperature).

From these results 15% sucrose was selected as the optimum concentration, but in subsequent experiments very little tube growth occurred after the first two hours.

B. *The method of La Cour and Faberge* (1943).

The pollen grains are grown on small squares of water permeable cellophane which is placed over a drop of sugar solution. These cellophane squares can be used for subsequent fixation and staining because the pollen tubes adhere quite firmly to them.

Four sugar concentrations were tried and the optimum sugar concentration was also combined with the addition of boric acid (200 p.p.m.). The results can be seen in the following table.

TABLE 2

Sucrose %	2 hours Sucrose only	2 hours + 200 p.p.m. boric acid	3 hours + 200 p.p.m. boric acid	12 hours Sucrose only
7	60μ	—	—	—
10	125μ	—	—	—
12	125μ	—	—	—
15	260μ	310μ	510μ	500μ

From the above table one can see that 15% sucrose again gave best pollen tube growth. After two hours the growth was slightly better than when the tubes are grown in sugar-agar medium, but subsequent pollen tube growth was much more successful on cellophane (500μ after 12 hours on cellophane compared with 240μ after 15 hours in sugar-agar medium).

This method thus proved to be better than the other (and also more convenient), and all subsequent work was done by germinating the pollen in 15% sucrose on cellophane paper, adding 200 p.p.m. boric acid.

C. *Staining and fixation.*

Fixation was done in Carnoy's Fluid for 15 minutes, and this was followed by orcein staining or by the Feulgen technique, staining for 4 hours in leucobasic Fuchsin to the recipe of Kaston and Burton (1959) and hydrolysing at 60°C for 7 minutes in 1 N HCl.

Colchicine and acenaphthene treatment.

The generative nucleus divides in the tube after 5 hours, but the chromosomes were grouped so closely together that metaphase analysis was practically impossible. It was therefore necessary to destroy the spindle in order to obtain greater spreading of chromosomes. For this reason 0·1%, 0·01%, 0·005% and 0·001% colchicine was used in the growing medium of 15% sucrose.

The best results were obtained in 0·005% colchicine, for which the following general observations were made: After six hours the chromosomes become denser but they are still grouped together (Fig. 1). Maximum spreading occurs after 11—18 hours (Fig. 2) and after 22 hours the chromosomes begin to despiralize and apparently form a restitution nucleus (Fig. 3).

The acenaphthene treatment did not give good results. The pollen was grown on sucrose–boric acid medium as described, and were kept in a closed petri dish in which 0·1 gm of acenaphthene was scattered. After 6 hours most of the nuclei were still in the pollen grains and the few nuclei which entered some tubes were still in prophase. This treatment resulted in inhibition, and was not continued.

RESULTS

1. *Hypoxis filiformis*, n = 7. The tip of the pollen tube often became swollen (Fig. 4*a*) but the chromosomes could often be observed very clearly (Figs. 4*b* and 4*c*), and the haploid number of 7 could be determined in these pollen tubes.

In Fig. 4*d*, the chromosome number is apparently 10, but here it is obvious that the four chromosomes to the left are double structures and that the six chromosomes to the right represent three chromosomes which of course were also double and in which the chromatids separated to form the six chromosomes observed. In the figure the dotted lines indicate the pairs which originally represented three chromosomes. This can be expected after prolonged colchicine treatment and must be considered a serious hazard when such determinations are made of high chromosome-number plants.

2. *Hypoxis sp.* (probably a form of *H. rooperi*). n = 43–58. Figs. 5*a* and 5*b*. From the discussion above it must be realised that counts of these high chromosome-number plants cannot be absolutely reliable. Counts varied between 43 and 58, and although it is agreed that these counts may to some extent be unreliable it is obvious that different generative nuclei from different pollen grains of one plant vary in chromosome number.

3. *Hypoxis acuminata*. n = 18 (20). Nine determinations could be made and in seven cases the number was 18 (e.g. Fig. 6*a* and 6*b*), but in two tubes the

number was 20, although every care was taken not to consider two separated chromatids as representing two separate chromosomes (Fig. 6*c*).

4. *Hypoxis sp.* n + 7. This plant could not be identified but the result is presented here because it duplicates the number which was also obtained for *H. filiformis*.

DISCUSSION

When these results are combined with the data presented in the previous paper, the following summary can be made:-

	Root tips	*Generative nuclei in pollen grains*
Hypoxis stellipilis	2n = 16	
,, *zeyheri*	2n = 32	
,, *multiceps*	2n = 36	
,, *longifolia*	2n = 72	
,, *rooperi*	2n = 96	n = ± 43—58
,, *acuminata*		n = 18 (20)
,, *sp.*		n = 7
,, *filiformis*		n = 7

The lowest chromosome number recorded is n = 7, but this cannot be accepted as the basic number of the genus without further study. There is definitely not a simple series of polyploidy in this genus.

The low chromosome number species (*H. filiformis*, *H. stellipilis* and *H. species*) are all relatively small plants compared with the much larger *H. longifolia* and *H. rooperi*, which have much higher chromosome numbers.

The meiosis of the 7 chromosome species is apparently quite normal (the number 7 is constantly present in all the pollen tubes) but the abnormal meiosis of the high chromosome species as described in the previous paper is confirmed here by the variation in chromosome number in the generative cell, and this apparently is also the case in species with haploid numbers as low as 18 (e.g. *H. acuminata*).

It seems very unlikely that such variation in pollen tubes and such abnormal meiosis would occur in populations which are reproducing sexually unless hybridization is taking place frequently or unless the plants are apomictic (which may of course involve a pre-history of hybridization). The morphological polymorphism of the bigger "species", their high ploidy and abnormal meiosis point to the likelihood that these plants are apomictic (see e.g. Stebbins, 1951). The smaller Hypoxis species on the other hand show much less morphological variation, the species seem much better defined and the meiosis is apparently

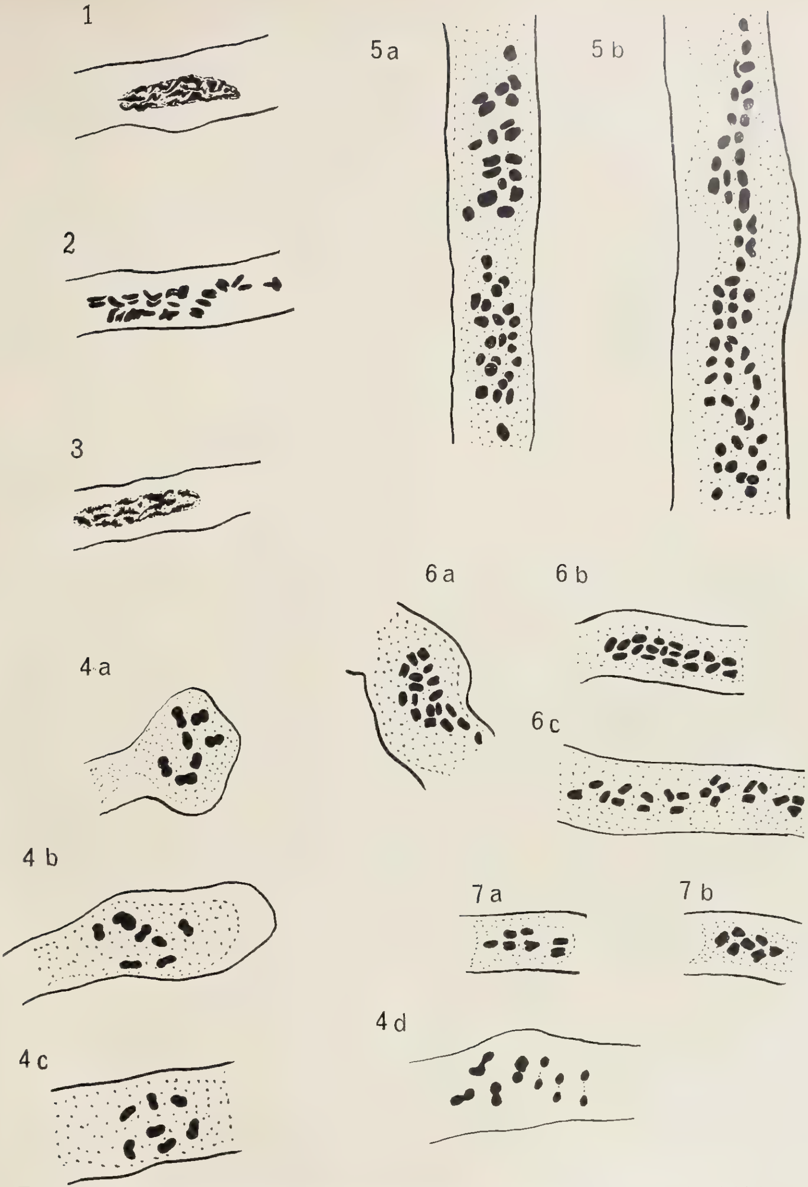

FIG. 1. *Hypoxis rooperi.* Pollen tubes grown in 15% sucrose with 0·005% colchicine. Nucleus in prophase after six hours. FIG. 2. Same after 11–18 hours. Some spreading occurs, but not sufficient for an accurate determination of chromosome number. FIG. 3. Same after 22 hours. A restitution nucleus is produced. FIG. 4. *Hypoxis filiformis:* n= 7 in Figs. a, b and c. In Fig. 4d the chromatids of three chromosomes have separated (indicated by dotted line). FIG. 5. *Hypoxis* species (similar to H. rooperii). n = 42 (a), n = 58 (b). FIG. 6. *Hypoxis acuminata:* a and b, n = 18; c, n = 20. FIG. 7. a and b, *Hypoxis* species. n = 7.

normal. From these data one can make the suggestion that they are reproducing sexually. These assumptions are tested in the next paper.

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